

## Remarks

### The Amendments

#### Claims

Claim 66 recites providing a sample comprising "X%" of a target nucleic acid and dividing the sample to produce a plurality of assay samples in which the ratio of target to non-target nucleic acid in at least one of the samples is greater than "X%." Claim 66 has been amended to include the recitation that "X is less than 100." The amendment is supported by the specification which discloses, "If the analysis method being used requires 100% analyte to detect, then dilution down to the single template molecule level will be required." (Page 9, lines 12-14.)

Claims 65, 66, and 67 have been amended include the recitations of claim 98.

New claims 110, 111, and 122 are claim 100 as it depended from claims 65, 66, and 67, respectively, rewritten in independent form.

Claim 68 has been amended to recite dependency on new claim 111 in addition to claim 66. Claims 70, 74-76, 89, 91, 92, 94-97, and 103 have been amended to recite dependency on new claims 110, 111, and 112 in addition to claims 65, 66, and 67. Claim 71 has been amended to recite dependency on new claims 110 and 112 in addition to claims 65 and 67. Claim 78 has been amended to recite dependency on new claim 110 in addition to claim 65. Claim 102 has been amended to recite dependency on new claims 111 and 112 in addition to claims 66 and 67. These amendments were made to make claims dependent on at least one of claims 65, 66, and 67 also dependent on corresponding claims 110, 111, and 112.

Claim 72 has been amended to recite dependency on claims 102 or 109 in place of claims 65, 66, or 67. The amendment provides antecedent basis for the "diluted" samples recited in claim 72.

Claim 93 has been amended to recite dependency on claims 65, 66, 67, 110, 111, or 112 in place of claim 90 and claim 98 has been amended to recite dependency on claims 110, 111, and 112 in place of claim 90, now canceled.

Claim 100 has been amended to recite dependency on claims 65, 66, and 67 in place of claim 89. Claim 100 does not properly refer to claim 89.

New claim 109 recites that the separating step of claim 65 "comprises diluting said sample." This amendment is supported by originally filed claim 102, which recites that samples are diluted.

New claim 113 recites that the sample of claim 109 is diluted so that between about 0.1 and 0.9 of the assay samples yield an amplification product when amplified. New claim 114 recites that the sample of claim 113 is diluted so that between about 0.1 and 0.6 of the assay samples yield an amplification product when amplified. These claims are supported by originally filed claims 72 and 73, which contain similar recitations.

New claim 114 recites that the first loop of the first molecular beacon probe in the method of claims 65, 66, or 67 "consists of 16 base pairs and has a  $T_m$  of 50-51°C" and the first stem of the first molecular beacon probe "consists of 4 base pairs having a sequence 5'-CACG-3'." Claim 114 is supported by originally filed claim 99, which contains these elements.

New claim 115 recites that the second loop of the second molecular beacon probe recited in claims 110, 11, or 112 "consists of 19-20 base pairs and has a  $T_m$  of 54-56°C" and

that the second stem of the second molecular beacon probe "consists of 4 base pairs having a sequence 5'-CACG-3'." Claim 115 is supported by originally filed claim 101, which recites these elements.

### Specification

The numbers in parentheses refer to the paragraphs as numbered in the "Amendments to the Specification."

(1) The specification has been amended to specify that the application is a "continuation of" in place of "claims priority to" application serial number 09/613,826. The specification has also been amended to provide the U.S. Patent Number that issued from application serial number 09/613,826.

(2), (3), and (9) The specification has been amended to include the degree symbol "°" in place of "□" immediately following each disclosed temperature.

(4) The specification has been amended to specify that Figure 1 includes 1A, 1B, and 1C.

(5), (6), (7), and (8) The specification has been amended to disclose the sequence identifier for each of the sequences described in the specification and figures.

(10) The specification has been amended to enter a substitute sequence listing. The substitute sequence listing includes sequences that were not present in the prior sequence listing. The additional sequences are disclosed in Figures 2, 4, and 5 of the drawings.

These amendments introduce no new matter.

### Sequence Compliance

The Office Action indicates that the patent application is objected for containing nucleotide and/or amino acid sequence disclosures but as not containing a sequence listing as required in 37 C.F.R. §§ 1.821-1.825.

A sequence listing was submitted with the application for patent filed October 12, 2001.

However, the sequence listing submitted with the application did not disclose the sequences in Figures 2, 4, and 5 of the drawings. Thus a substitute sequence listing accompanies this response. A computer readable form of the substitute sequence listing is also enclosed. I believe that the paper and computer readable form of the sequence listing are identical in content. The substitute sequence listing introduces no new matter.

Withdrawal of this objection is respectfully requested.

### Priority

The Office Action has objected to the specification of the application as not properly referring to previously filed applications to which it claims priority.

The specification has been amended to indicate that the instant application is "a continuation of" in place of "claims priority to" application serial number 09/613,826. The specification has also been amended to provide the U.S. Patent Number that issued from application serial number 09/613,826.

Withdrawal of this objection is respectfully requested.

### Specification

The specification is objected to as lacking the degree symbol. The degree symbol has been added at the appropriate paragraphs of the specification.

The specification is also objected to as not containing sequence identifiers following sequences that are disclosed in the sequence listing. Sequence identifiers have been added following the sequences disclosed in the sequence listing.

Withdrawal of these objections is respectfully requested.

### The Rejection of Claims 66-77, and 89-103 Under 35 U.S.C. § 112, Second Paragraph

Claims 66-77 and 89-103 have been rejected under 35 U.S.C. § 112, second paragraph as indefinite. The Office Actions lists five specific reasons why the claims are allegedly indefinite. Each will be discussed in turn.

1. The Office Action alleges that claims 98 and 99 are indefinite because the recitation "the first detectable probe" lacks antecedent basis. Claim 98 has been amended to delete the recitation "the first detectable probe." Thus the rejection is rendered moot.
2. The Office Action alleges that claims 100 and 101 are indefinite because the recitation "the second detectable probe" lacks antecedent basis. Claims 100 and 101 have been amended to delete the recitation "a second detectable probe." Thus the rejection has been rendered moot.
3. The Office Action asserts that claim 102, as it depends from claim 65, lacks antecedent basis for the recitation "said dividing step." Claim 102 has been amended to delete the dependency on claim 65. Thus this deficiency has been corrected.

4. The Office Action asserts that claims 66, 68-77, and 89-103 are indefinite because it is unclear what range is covered by the recitation "X%." Claim 66 has been amended to recite that "X is less than 100." Thus the range of percentages recited in claim 66 and dependent claims 68-77, and 89-103 is clear.

5. The Office Action asserts that claim 67 is indefinite because it is unclear whether "the single target nucleic acid" recited in the step of amplifying refers to any single target nucleic acid within the heterogeneous sample or to a single target nucleic acid present in one of the plurality of samples from which the heterogeneous sample was divided. Claim 67 has been amended to recite that dividing heterogeneous samples produces divided samples and that at least one of the divided samples comprises "a single molecule of target nucleic acid." Thus "the single molecule of the target nucleic acid" that is subsequently amplified is a single molecule of target nucleic acid present in one of the plurality of assay samples from which the heterogeneous sample was divided. Thus this deficiency has been corrected.

Withdrawal of these rejections to claims 66-77 and 89-103 is respectfully requested.

The Rejections of Claims 65-73, 76-78, 90, 91, 102, and 103 Under 35 U.S.C. § 102(b)

The Office Action has rejected claims 65-73, 76-78, 90, 91, 102, and 103 as anticipated by either Bunn *et al.* (U.S. Patent 5,213,961) or Kramer *et al.* (WO99/13113).

- Claims 65-73, 76-78, 91, 102, and 103 have been rejected under 35 U.S.C. § 102(b) as anticipated by Bunn *et al.* (U.S. Patent 5,213,961).
- Claims 65-71, 90, 91-93, and 103 have been rejected under 35 U.S.C. § 102(b) as being anticipated by Kramer *et al.* (WO99/13113).

"A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631 (Fed. Cir. 1987).

Amended claims 65-67 are the only independent claims of the rejected claim set. Each recites a method for detecting a target nucleic acid. The target nucleic acid is present in a sample. The sample is separated or divided, the target nucleic acid is amplified, the amplified product is hybridized to first and second molecular beacon probes, and the probes hybridized to the target nucleic acid are detected. The first molecular beacon probe hybridizes to the target nucleic acid. The first molecular beacon probe comprises "a first stem which comprises about 4 base pairs having a sequence 5'-CACG-3', and a first loop structure which comprises about 16 base pairs and has a  $T_m$  of about 50-51°C."

Neither Bunn nor Kramer expressly or inherently teaches such a probe. The Office Action acknowledges that Bunn and Kramer lack this teaching:

There is no prior art that teaches or suggests a molecular beacon probe that has a loop consisting of 16 base pairs and a  $T_m$  of 50-51°C with a stem consisting of CACG sequence. . . . The closest prior art is Tyagi et al (US5,925,517) who teaches a molecular beacon with has a 15 base pair loop but a  $T_m$  of approximately 40°C ( $T_m = [(A+T) \times 2C + (G+C) \times 4C]$ ) and the stem is GCGAG. Tyagi et al (US6,037,130) [also] teaches a molecular beacon with a stem comprising CACG but with a loop of  $T_m$  65°C.

Paper 8, page 13, lines 12-19.

Thus Bunn does not expressly or inherently teach each and every element of claims 65-67. Kramer also does not expressly or inherently teach each and every element recited in claims 65-67. Claims 68-73, 76-78, 90, 91, 102, and 103 depend from claims 65-67 and

thus also recite this element. Withdrawal of the anticipation rejections is respectfully requested.

The Rejection of Claims 74, 75, 79-89, and 94-97 Under 35 U.S.C. § 103(a)

Claims 74, 75, 79-89, and 94-97 have each been rejected under 35 U.S.C. § 103(a) as being unpatentable over Bunn *et al.* or Kramer *et al.* in view of a secondary reference.

- Claims 75 and 95 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Bunn *et al.* or Kramer *et al.* in view of Gruenert *et al.* (U.S. 5,804,383).
- Claims 96 and 97 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Bunn *et al.* or Kramer *et al.* in view of Sidransky *et al.* (U.S. 5,518,901).
- Claim 89 has been rejected under 35 U.S.C. § 103(a) as being unpatentable over Bunn *et al.* or Kramer *et al.* in view of Schmitt (*Forensic Science International* (1994) 66:129-141).
- Claims 79-88 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Bunn *et al.* or Kramer *et al.* in view of Livak *et al.* (U.S. 5,736,333).
- Claim 74 has been rejected under 35 U.S.C. § 103(a) as being unpatentable over Bunn *et al.* or Kramer *et al.* in view of Ruano *et al.* (*Proc. Acad. Sci. USA* (1990) 87:6296-6300).
- Claim 94 has been rejected under 35 U.S.C. § 103(a) as being unpatentable over Bunn *et al.* or Kramer *et al.* in view of Murtagh (U.S. 5,518,901).

To establish *prima facie* obviousness of a claimed invention, all the claim limitations must be taught or suggested by the prior art. *In re Royka*, 490 F.2d 981 (CCPA 1970).

Each of the dependent claims of the rejected claim set depends from claims 65, 66, and 67. Amended claims 65, 66, and 67 recite detecting a target nucleic acid using a first molecular beacon probe. The first molecular beacon probe comprises "a first stem which comprises about 4 base pairs having a sequence 5'-CACG-3', and a first loop structure which comprises about 16 base pairs and has a  $T_m$  of about 50-51°C."



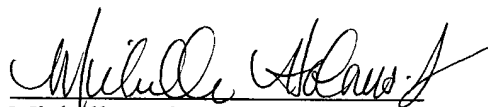
The combination of Bunn or Kramer with any of secondary references Gruenert, Sidransky, Schmitt, Livak, Ruano, or Murtagh does not teach or suggest the recited first molecular beacon probe. The Patent Office acknowledges that the prior art, including the cited references, does not teach or suggest this first molecular beacon probe. (Paper 8, page 13, lines 12-14.)

Thus Bunn or Kramer in combination with Gruenert, Sidransky, Schmitt, Livak, Ruano, or Murtagh does not teach or suggest all elements of amended claims 65-67. Claims 74, 75, 79-89, and 94-97 depend from claims 65-67 and thus also contain this recitation. Withdrawal of the obviousness rejections to claims 74, 75, 79-89, and 94-97 is respectfully requested.

Respectfully submitted,

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